Attachment # 1

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Citizen Petition to Establish Safety of Echinacea Extract as a GRAS Pharmaceutical Formulation Aid – CMC Section

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Table of Contents

List of Tables

List of Figures

1. Introduction

In the last few years, we have been facing a worldwide trend toward comsuming more natural / herbal nutrtional supplements than synthetic / chemical compounds. The same trend is affecting the medicinal drug industry and specifically the over-the-counter (OTC) drug market. The intention is to replace synthetic pharmaceutical excipients with natural herbal food additives. Echinacea, as a technical formulation aid, has been found to inherently possess binding properties, which can be utilized in pharmaceutical formulations as a substitute for chemicals including: povidone, polyethylene glycol, cellulose deviatives, etc. Additionally, Echinacea is a natural colorant replacing the need to incorporate synthetic dyes in medicinal formulations.

Echinacea is a plant that grows 1-2 feet tall and has a spiny appearance from which it derived its name. The plant is a member of the daisy family and has a faint aromatic smell. It grows throughout the United States, but is primarily found in states from the Midwest to the prairie regions of Pennsylvania. There are several different species of Echinacea – the most common are E. angustifolia, E. pallida and E. purpurea. It is a very popular herb in the United States and Europe and can be found alone or in combination with many herbal nutritional supplements.

This document presents evidence that substantiates the formulation of pharmaceutical solid dosage forms using Echinacea instead of currently accepted US Food and Drug Administration (FDA) Generally Accepted As Safe (GRAS) ingredients as a technical formulation aid (binding agent).

2. Procedure

Several experiments were conducted to evaluate the feasibility of using Echinacea as a binding agent in various solid dosage forms. Active materials were chosen from among popular OTC and prescription drugs and forms of presentations were chosen to cover a variety of common solid dosage forms. Experiments identified as Numbers 1 and 2 examined the formulation of placebo tablets with echinacea as the binding agent. Experiments 3,4 and 5 examined the formulation of several OTC drug products. In addition, several types of manufacturing technologies were employed to fully evaluate the capacity of echinacea for its intended use. All research and development activities were performed at the CTS Chemical Industries Ltd. facilities in Israel.

2.1 Raw materials

2.1.1 Active Materials

Table 1 presents active ingredients used in these experiments. In general, materials should comply with relevant USP/NF monographs and suppliers must conform to FDA Good Manufacturing Practice (GMP) regulations.

Table 1: Active Materials Used in Binding Study Experiments

Material	Specifications	Experiment No.
Acetaminophen	USP	3
Loratadine	In House	4
Dextromethorphan Hydrobromide	USP	5

2.1.2 Inactive Materials

Table 2 presents inactive ingredients used in these experiments. In general, materials should comply with relevant USP/NF monographs and suppliers must conform to FDA GMP regulations.

Table 2: Inactive Materials Used in Binding Study Experiments

Material	Purpose	Specifications	Experiment Number
Polyethylene Glycol 6000	binder	NF	1
Polyethylene Glycol 4000	plastisizer	NF	5
Lactose	filler	NF	1,2,4
Magnesium Stearate	lubricant	NF	1,2,3,4
Purified Water	wetting agent	USP	1,2,3, 5
Ethanol	wetting agent	USP	1,2
Sodium Starch Glycolate	disintegrant	NF	3
Sugar Spheres	neutral carrier	NF	5

2.1.3 Echinacea Specifications

Table 3 presents material specifications for Echinacea used in these experiments. The May-June 2000 issue of the Pharmacopeial Forum

introduced proposed monographs for Echinacea to be included in the next edition of the NF. Copies of these monographs are presented in Appendix A. A copy of a supplier's Certificate of Analysis is presented in Appendix B. In general, we intend to comply with relevant NF monographs and FDA GMP regulations and our suppliers will conform to FDA GMP regulations. Dry and liquid extracts of E. purpurea have been used in these experiments. Extracts of E. angustifolia and E. pallida extracts may be used as well.

Table 3: Material Specifications for Powdered Echinacea Purpurea Extract

Test	**Specification**
Description	Brown, fine powder
Identification	Complies with retention time of standard from HPLC assay
Loss on drying	NMT 10.0%
Pesticide residues	Complies to purity data sheet
Heavy metals	NMT 0.001%
Microbial limits: Aerobic bacteria Fungi Enterobacter and gram-negative bacteria E. coli Staphylococcus aeruginosa Salmonella	 < 1000 cfu/g < 1000 cfu/g < 1000 cfu/g absent absent absent
Assay: Content of Fructofuranosid	 NLT 4.0%

2.1.4 Technologies

Several manufacturing technologies were evaluated to establish binding properties of Echinacea. These are:

- Wet granulation by Fluidized bed technology (using Glatt equipment) where Echinacea solution was sprayed on fluidized powder mix.
- Wet granulation by high sheer mixer where Echinacea solution was mixed with powders.
- Dry granulation where Echinacea powder was mixed with other powders without wetting.
- Pellet preparation using Wurster technology where active material is suspended in Echinacea binding solution and sprayed on neutral sugar spheres.

2.2 Formulations and Manufacturing Procedures

2.2.1 Experiment 1

Purpose:
Purposes of this experiment were twofold. The first was to evaluate the binding efficiency of Echinacea in placebo tablets prepared by wet granulation. The second was to compare results of tablets prepared with Echinacea to tablets prepared without Echinacea.

Procedure:
Placebo tablets were prepared according to a standard formula containing a weak binder (Polyethylene Glycol 6000) compared with the same composition plus Echinacea.

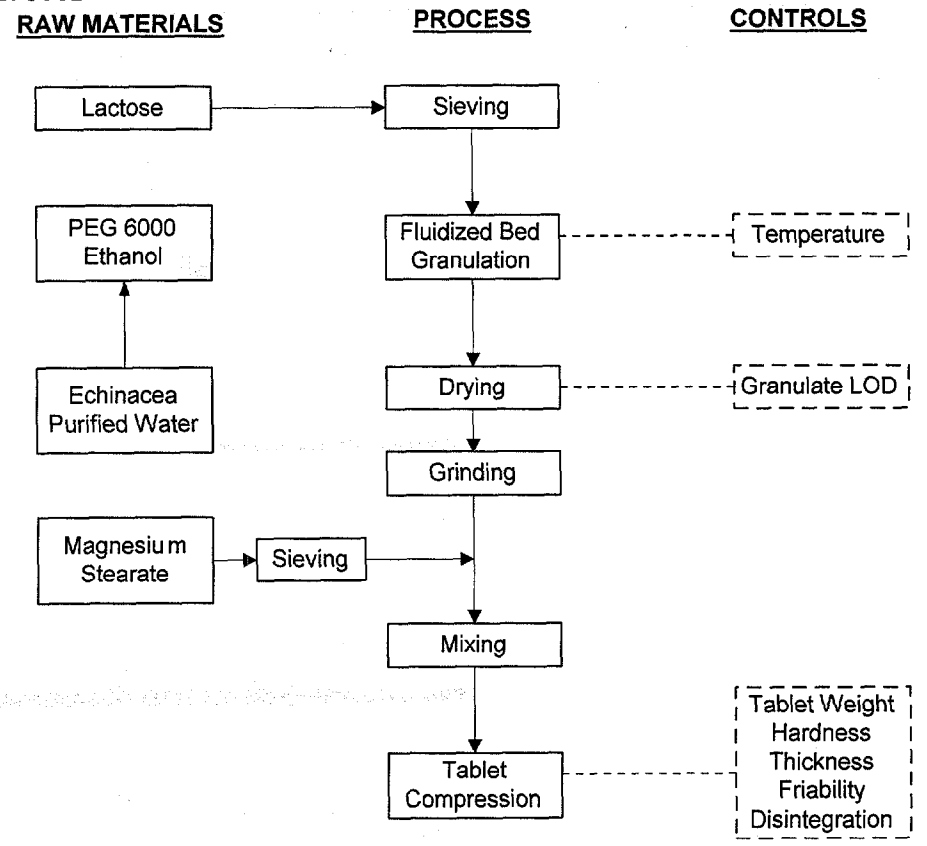
Formulations:
Refer to Table 4

Table 4: Placebo Formulations With and Without Echinacea as a Binding Agent

Ingredient	**Ingredient Purpose**	**Batch No. 270591 (mg/tab)** (Without Echinacea)	**Batch No. 270592 (mg/tab)** (With Echinacea)
Lactose	filler	94.2	91.2
Polyethylene Glycol 6000	binder	5.0	5.0
Echinacea	binder	-	3.0
Magnesium Stearate	lubricant	0.8	0.8

Manufacturing method:
Shown in Figure 1

Figure 1: Manufacturing and In-process Control for Batches No.270591and 270592



Results:

The comparative particle size distributions of granulates are shown in Table 5 and Figure 2. It can be seen that normal gausian distributions with similar means are obtained. These results indicate that Echinacea does not change granulate particle size distribution in similar formulations prepared with and without this ingredient.

Table 5: Particle Size Analysis for Batch No. 270591 (Without Echinacea) and 270592 (With Echinacea)

Batch No. 270591

	SIEVE OPENING (μ)	MEAN PARTICLE SIZE (μ)	TARE WEIGHT (g)	TOTAL WEIGHT (g)	NET WEIGHT (g)	PERCENT %	CUMULATIVE PERCENT %
BOTTOM	0	37.5	332.0	335.2	3.2	3.14	3.14
200 MESH	75	112.5	294.3	311.4	17.1	16.77	19.91
100 MESH	150	181	291.2	323.1	31.9	31.28	51.19
70 MESH	212	256	288.4	325.0	36.6	35.83	87.02
50 MESH	300	362.5	309.2	320.2	11.0	10.78	97.80
40 MESH	425	512.5	327.2	329.0	1.8	1.80	99.61
30 MESH	600	725	336.4	336.6	0.2	0.20	99.80
20 MESH	850	925	369.4	369.6	0.2	0.20	100.00
18 MESH	1000	> 1000	350.1	350.1	0.0	0.00	100.00
TOTAL					**102.0**		

Mean Particle Size : 220.0 micron

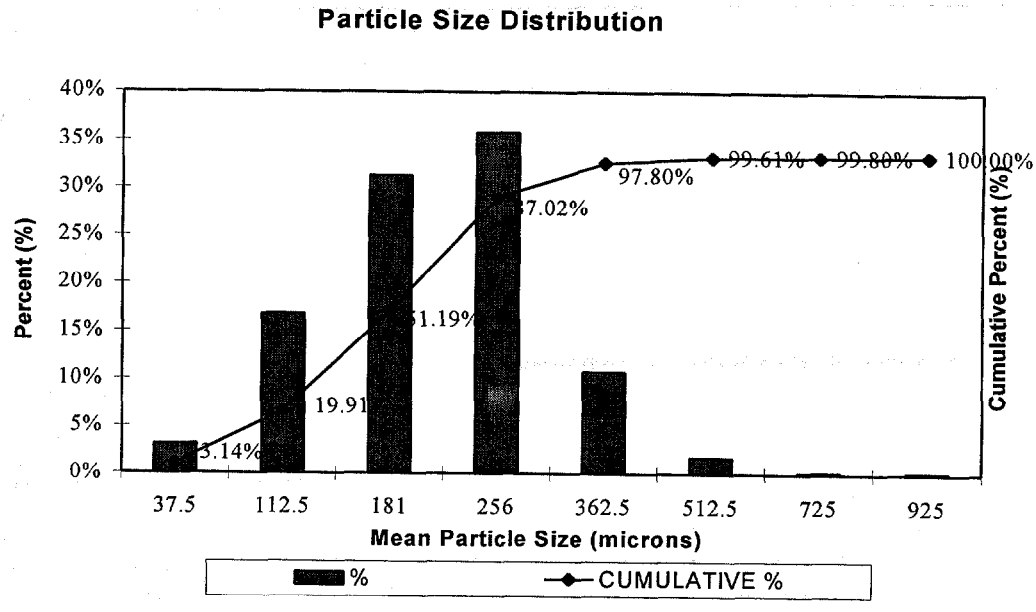
Batch No. 270592

	SIEVE OPENING (μ)	MEAN PARTICLE SIZE (μ)	TARE WEIGHT (g)	TOTAL WEIGHT (g)	NET WEIGHT (g)	PERCENT %	CUMULATIVE PERCENT %
BOTTOM	0	37.5	332.0	334.2	2.2	2.23	2.23
200 MESH	75	112.5	294.3	311.4	17.1	17.01	19.24
100 MESH	150	181	291.2	323.6	32.4	32.21	51.45
70 MESH	212	256	288.4	323.0	34.6	34.44	85.88
50 MESH	300	362.5	309.2	321.8	12.6	12.53	98.41
40 MESH	425	512.5	327.2	328.8	1.6	1.59	100.00
30 MESH	600	725	336.4	336.4	0.0	0.00	100.00
20 MESH	850	925	369.4	369.4	0.0	0.00	100.00
18 MESH	1000	> 1000	350.1	350.2	0.1	0.10	100.10
TOTAL					**100.6**		

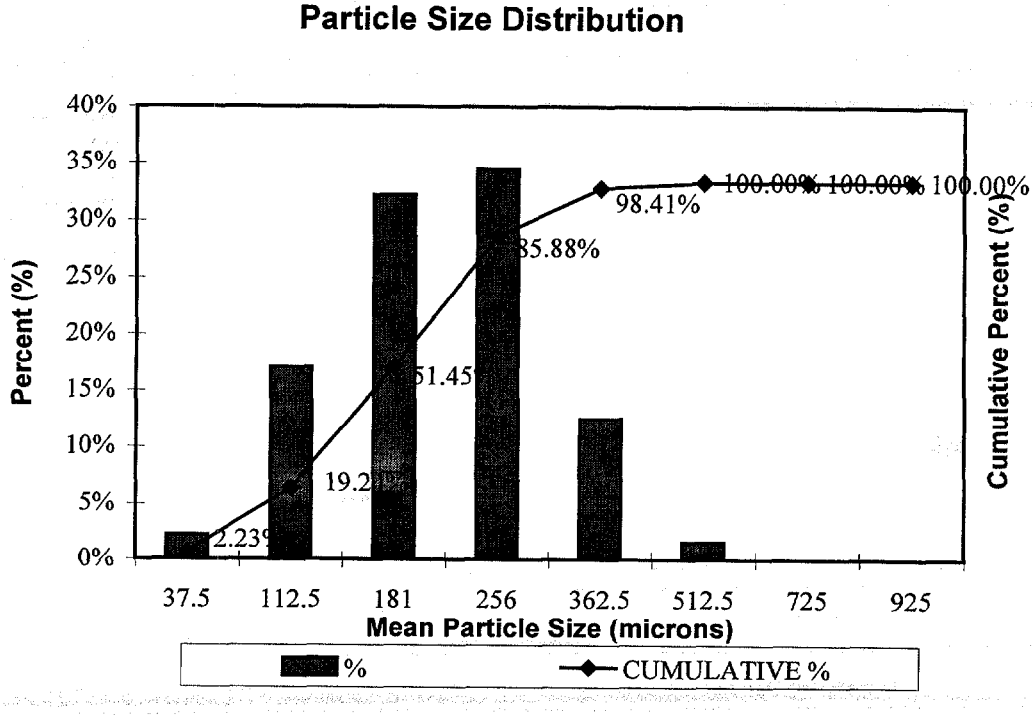
Mean Particle Size : 220.0 micron

Figure 2: Particle Size Distribution Analysis for Batch No. 270591 and 270592

Batch No. 270591



Batch No. 270592



Comparative tablets' parameters are presented in Table 6. Tablets compressed on the same machine using similar settings resulted in different tablet strengths while all other tablet parameters were not changed (weight, disintegration, friability). This indicates that Echinacea has binding properties.

Table 6: Placebo Formulations With and Without Echinacea as a Binding Agent – Finished Tablet Parameters

Test	Batch No. 270591	Batch No. 270592
Description	6 mm Normal Concave	6 mm Normal Concave
Average weight (mg/tab)	101.1	99.7
Hardness (Kp)	4.85	6.70
Disintegration (min)	9:18	9:42
Friability (%)	0.26	0.18

2.2.2 Experiment 2

Purpose:
The purpose of this experiment was to evaluate the binding efficiency of Echinacea in placebo tablets prepared by wet granulation.

Procedure:
Placebo tablets were prepared according to a standard formula containing Echinacea as a binder.

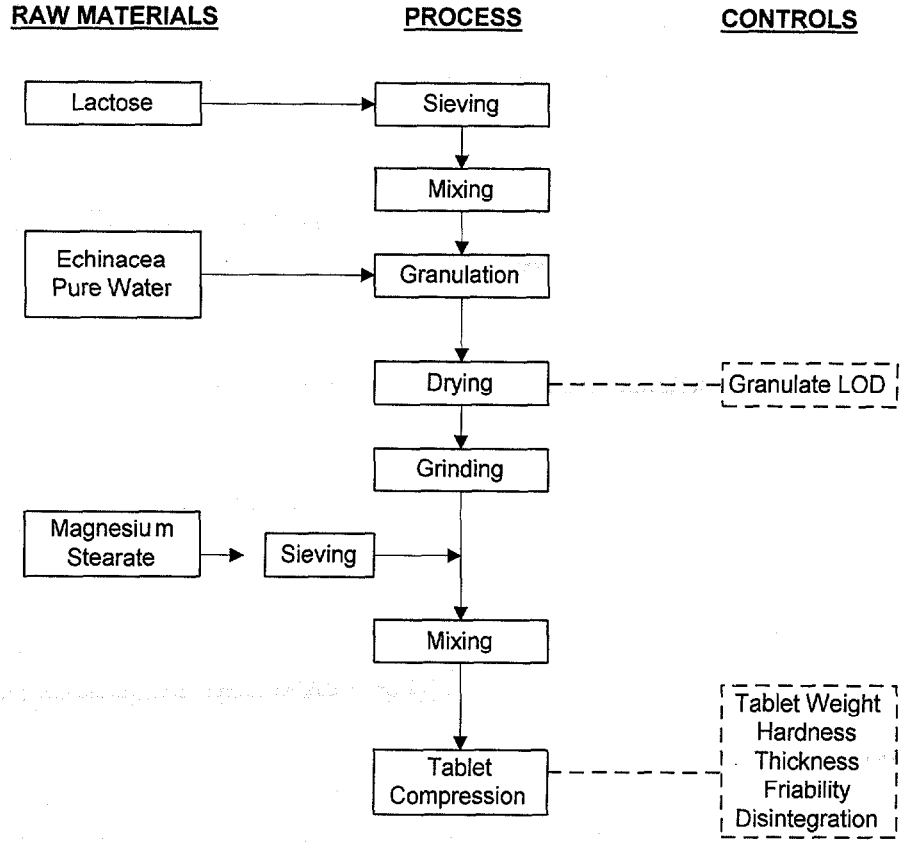
Formulation:
Described in Table 7

Table 7: Placebo Formulation with Echinacea as a Binding Agent - Batch No. 080691

Ingredient Name	Ingredient Purpose	Quantity (mg/tab)
Lactose	filler	124.5
Echinacea	binder	4.4
Magnesium Stearate	lubricant	1.1

Manufacturing method:
Shown in Figure 3

Figure 3: Manufacturing and In-process Control for Batch No. 080691



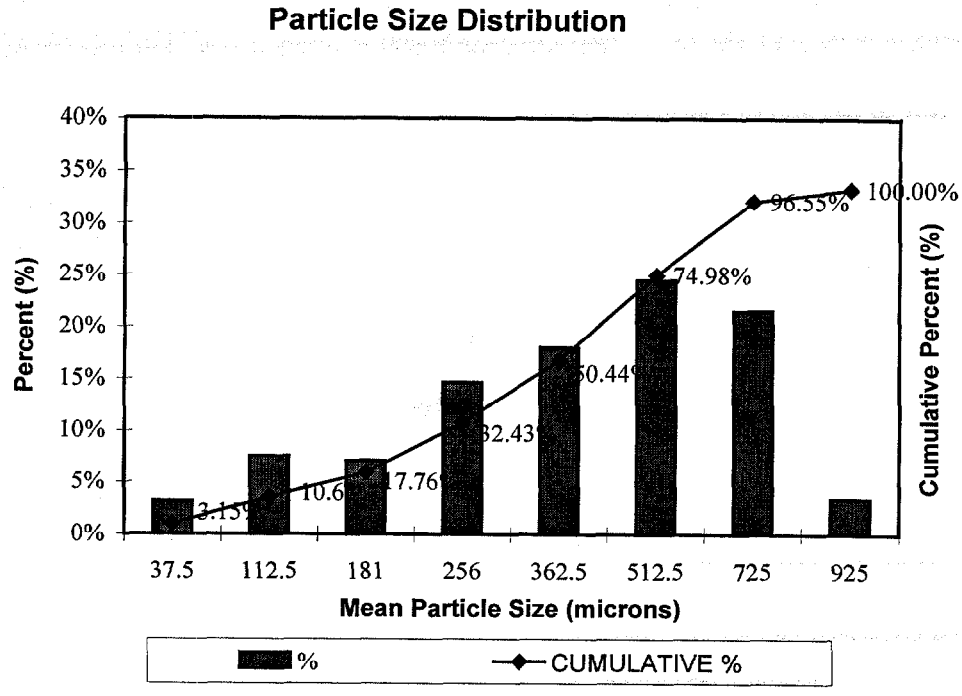
Results:
Particle size distributions of the granulate are shown in Table 8 and Figure 4. It can be seen that normal gausian distribution is obtained as expected from a regular wet granulation.

Table 8: Particle Size Analysis for Batch No. 080691

	SIEVE OPENING (μ)	MEAN PARTICLE SIZE (μ)	TARE WEIGHT (g)	TOTAL WEIGHT (g)	NET WEIGHT (g)	PERCENT (%)	CUMULATIVE PERCENT (%)
BOTTOM	0	37.5	332.0	334.8	2.8	3.15	3.15
200 MESH	75	112.5	294.3	301.1	6.8	7.51	10.65
100 MESH	150	181	291.2	297.6	6.4	7.11	17.76
70 MESH	212	256	288.4	301.6	13.2	14.67	32.43
50 MESH	300	362.5	309.2	325.4	16.2	18.02	50.44
40 MESH	425	512.5	327.2	349.3	22.1	24.53	74.98
30 MESH	600	725	336.4	355.8	19.4	21.57	96.55
20 MESH	850	925	369.4	372.5	3.1	3.45	100.00
18 MESH	1000	> 1000	350.1	350.2	0.1	0.11	100.11
TOTAL					**89.9**		

Mean Particle Size : **439.4 micron**

Figure 4: Particle Size Distribution Analysis for Batch No. 080691



Tablet parameters are presented in Table 9. It can be seen that physically acceptable tablets (more than 3-4 Kp) can be produced using Echinacea as a binder. No additional binder was required.

Table 9: Placebo Formulation with Echinacea as a Binding Agent – Finished Tablet Parameters for Batch No. 080691

Test	Results
Description	7 mm Flat Bevelled
Average weight (mg/tab)	129.0
Hardness (Kp)	8.49
Friability (%)	0.10

2.2.3 Experiment 3

Purpose:
The purpose of this experiment was to evaluate the binding efficiency of Echinacea in acetaminophen tablets prepared by wet granulation.

Procedure:
Acetaminophen 500mg tablets were prepared according to a typical formulation except for the replacement of povidone with Echinacea.

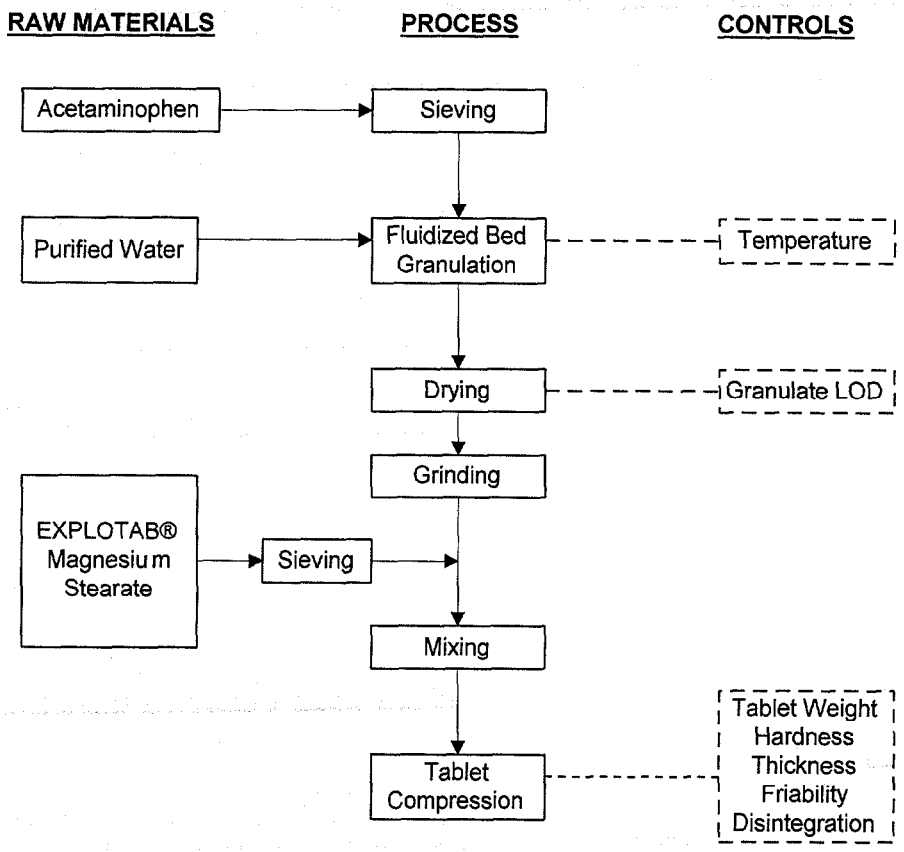
Formulation:
Described in Table 10.

Table 10: Acetaminophen Formulation with Echinacea as a Binding Agent - Batch No. 140791

Ingredient Name	Ingredient Purpose	Quantity (mg/tab)
Acetaminophen	active	500.0
Echinacea	binder	27.0
Sodium Starch Glycolate	disintegrant	21.0
Magnesium Stearate	lubricant	4.5

Manufacturing method:
Shown in Figure 5

Figure 5: Manufacturing and In-process Control for Batch No. 140791



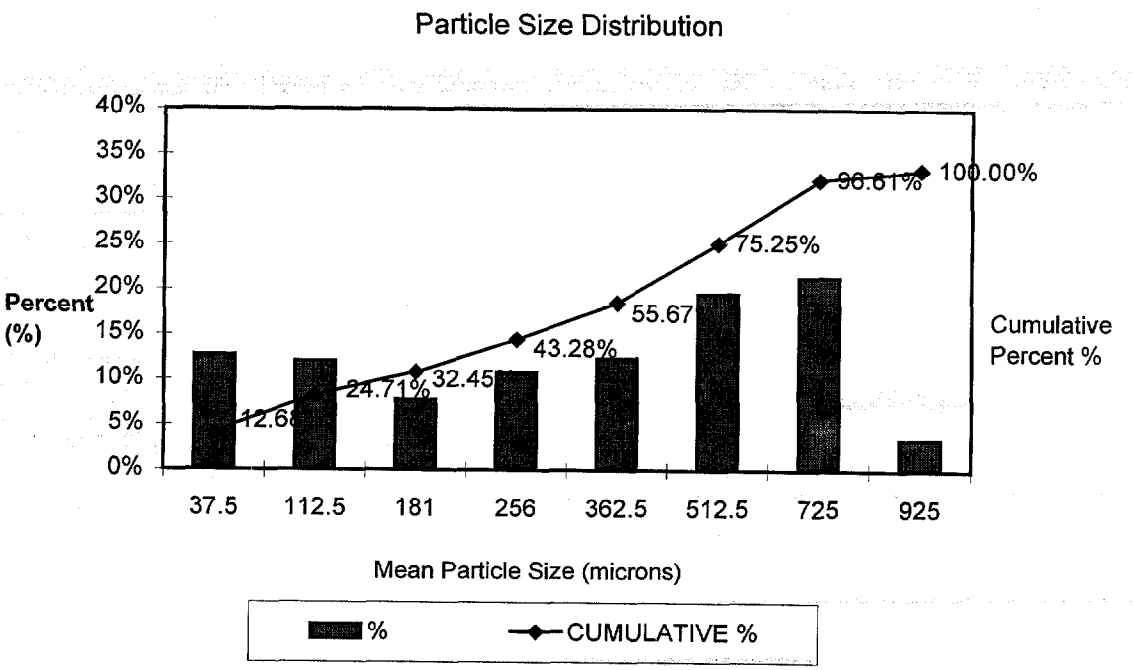
Results:
Particle size distributions of the granulate are shown in Table 11 and Figure 6. It can be seen that a normal distribution is obtained as expected from a regular wet granulation.

Table 11: Particle Size Analysis for Batch No. 140791

	SIEVE OPENING (μ)	MEAN PARTICLE SIZE (μ)	TARE WEIGHT (g)	TOTAL WEIGHT (g)	NET WEIGHT (g)	PERCENT (%)	CUMULATIVE PERCENT (%)
BOTTOM	0	37.5	332.0	338.2	6.2	12.68	12.68
200 MESH	75	112.5	294.3	300.2	5.9	12.03	24.71
100 MESH	150	181	291.2	295.0	3.8	7.74	32.45
70 MESH	212	256	288.4	293.7	5.3	10.83	43.28
50 MESH	300	362.5	309.2	315.3	6.1	12.39	55.67
40 MESH	425	512.5	327.2	336.8	9.6	19.59	75.25
30 MESH	600	725	336.4	346.9	10.5	21.35	96.61
20 MESH	850	925	369.4	371.1	1.7	3.39	100.00
18 MESH	1000	> 1000	350.1	350.63	0.5	1.08	101.08
TOTAL					**49.2**		

Mean Particle Size : 391.5 micron

Figure 6: Particle Size Distribution Analysis for Batch No. 140791



Tablet parameters are presented in Table 12. It can be seen that physically acceptable tablets can be produced using Echinacea as a binder. No additional binder was required.

Table 12: Acetaminophen Tablets with Echinacea as a Binding Agent – Resulting Tablet Parameters for Batch No. 140791

Test	Results
Description	13 mm Normal Concave
Average weight (mg/tab)	586.5
Disintegration (min)	2:13
Hardness (Kp)	3.46

Specifications:
Product specifications are presented in Table 13.

Table 13: Acetaminophen Tablets with Echinacea as a Binding Agent – Proposed Product Specifications

Acetaminophen Tablets			
No.	**Test Parameter**	**Specifications**	**Method**
1	Appearance	13mm, concave white tablets	Visual
2	Identification	Meet requirements	USP
3	Average Weight (mg/tablet) Range: 95-105%	552.5 524.9-580.1	USP
4	Uniformity of dosage unit	meet requirements	USP
5	Hardness (Kp)	NLT 2	In house
6	Disintegration (min)	NMT 15	USP
7	Friability (%)	NMT 1.0	USP
8	Assay (mg) Range: 90.0 % - 110.0%	500.0 450.0-550.0	USP
9	Dissolution	NLT 80%(Q) in 30 min	USP

2.2.4 Experiment 4

Purpose:
The purpose of this experiment was to evaluate the binding efficiency of Echinacea in Loratadine tablets using direct compression technology.

Procedure:
Loratadine 10mg and 20 mg tablets were prepared with Echinacea as a dry binder. The same powder mix was used to compress both tablet strengths.

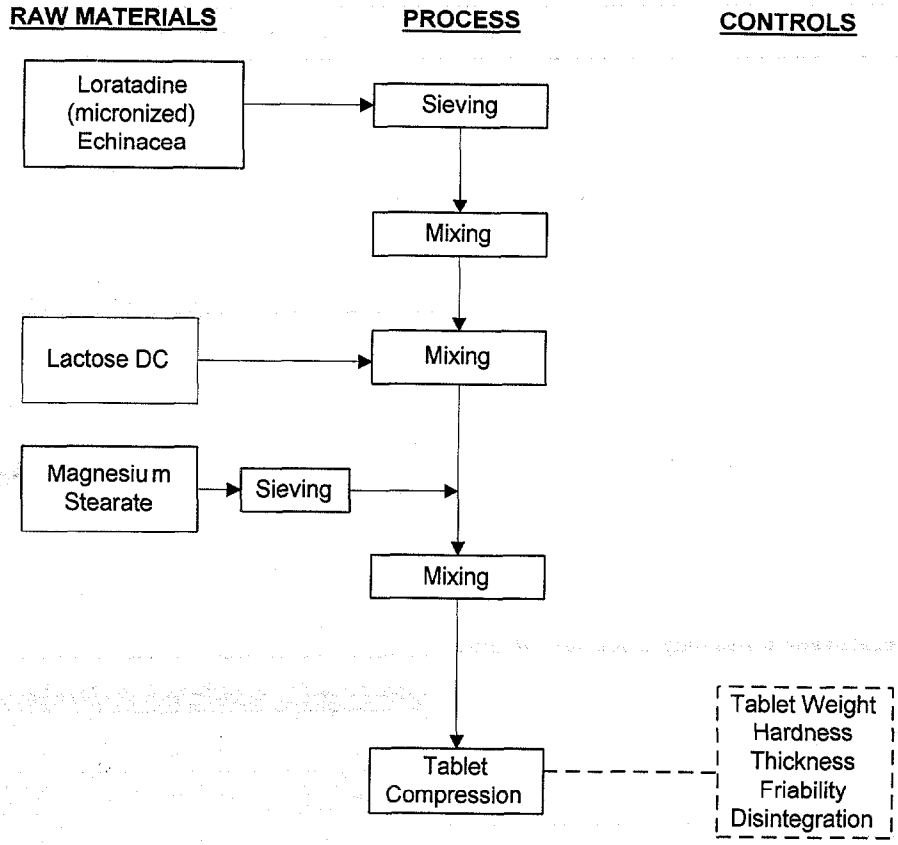
Formulation:
Described in Table 14.

Table 14: Loratadine Formulation with Echinacea as a Binding Agent - Batch No. 050794A (10 mg) and 050794B (20 mg)

Ingredient Name	**Ingredient Purpose**	**Quantity (mg/tab)**	
Loratadine	active	10.0	20.0
Echinacea	binder	25.0	50.0
Lactose	filler	163.0	326.0
Magnesium Stearate	lubricant	2.0	4.0

Manufacturing method:
Shown in Figure 7

Figure 7: Manufacturing and In-process Control for Batch No. 050794 A and B



Results:
Tablet parameters are presented in Table 15. The same powder mix was used to compress both strengths. It can be seen that physically acceptable tablets can be obtained using Echinacea as a dry binder. No additional binder was required.

Table 15: Loratadine 10mg and 20mg tablets with Echinacea as a Binding Agent – Resulting Tablet Parameters for Batch No. 050794A (10 mg) and 050794B (20 mg)

Test	**Batch No. 050794A**	**Batch No. 050794B**
Description	8 mm Normal Concave	10 mm Normal Concave
Average weight (mg/tab)	202.3	401.7
Friability (%)	0.19	0.23
Hardness (Kp)	9.90	8.51

Specifications:
Product specifications for the 10mg dosage form are presented in Table 16.

Table 16: Loratadine Tablets with Echinacea as a Binding Agent – Proposed Product Specifications

LORATADINE 10mg Tablets			
No.	**Test Parameter**	**Specifications**	**Method**
1	Appearance	8mm, concave white tablets	Visual
2	Identification	The retention time of peak in the chromatogram of the Assay preparation corresponds to that of the standard preparation as obtained in the Assay	In house
3	Average Weight (mg/tablet) Range: 92.5 - 107.5%	200.0 185.0-215.0	USP
4	Weight Variation	Meet requirements	USP
6	Hardness (Kp)	NLT 5	In house
7	Disintegration (min)	NMT 15	USP
8	Friability (%)	NMT 1.0	USP
9	Assay (mg) Range: 95.0 % - 105.0%	10.00 9.50 - 10.50	In house
10	Impurities and Degradation Products Determination (%) a. Each individual Impurity b. Total Impurities	 a. NMT 1.0 b. NMT 2.0	In house
11	Dissolution	NLT 80%(Q) in 30 min	In house

2.2.5 Experiment 5

Purpose:
The purpose of this experiment was to evaluate the binding efficiency of Echinacea in dextromethorphan pellets (microgranules).

Procedure:
Dextromethorphan 30 mg capsules were prepared with Echinacea.

Formulation:
Described in Table 17.

Table 17: Dextromethorphan Formulation with Echinacea as a Binding Agent - Batch No. 010791

Ingredient Name	Ingredient Purpose	Quantity (mg/g)
Dextromethorphan	active	30.0
Echinacea	binder	6.0
Polyethylene Glycol 4000	plastisizer	3.0
Sugar Spheres	carrier	561

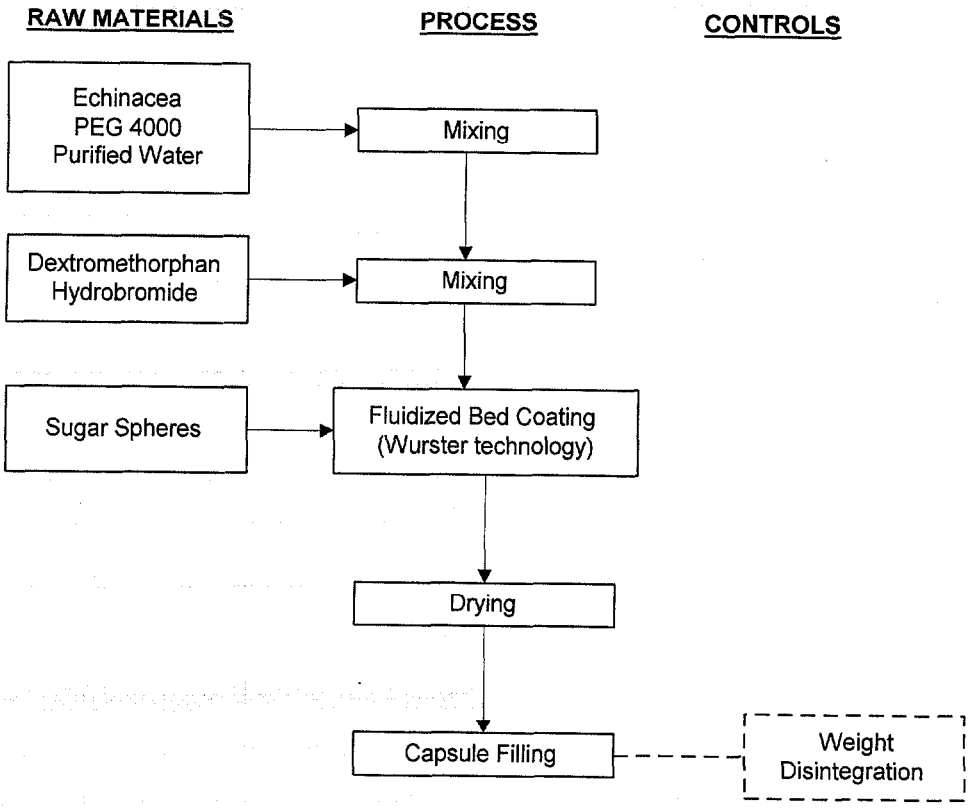
Specifications:
Product specifications are presented in Table 18.

Table 18: Dextromethorphan Formulation with Echinacea as a Binding Agent – Proposed Product Specifications

Dextromethorphan 30 mg Capsules			
No.	**Test Parameter**	**Specifications**	**Method**
1.	Description a) Capsule content b) Outer capsule	 Beige to greenish spherical pellets Hard gelatin capsule Cap: dark blue Body: pink	Visual
2.	Identification by HPLC	Rt of sample is concordant with Rt of standard (acc. to assay)	In house
3.	Average Fill Weight (mg/cap) Range: 90.0-110.0	200.0 220.0-180.0	USP
4.	Uniformity of dosage unit	Meet requirements	USP
5.	Assay (mg/cap) Range: 95.0 % - 105.0%	10.0 9.5-10.5	In house
6.	Dissolution (%)	NLT 80%(Q) in 30 min	In house

Manufacturing method:
Shown in Figure 8

Figure 8: Manufacturing and In-process Control for Batch No. 010791



3. Echinacea Quantities

The experiments shown above indicate that the amounts of Echinacea required to produce physically satisfactory pharmaceutical solid dosage forms are below 30mg/unit dose. The upper limit of the maximum amount required as a technical formulation aid is significantly below the recommended dosage of Echinacea in herbal supplements containing 150-300 mg per tablet.

4. Finished Product Release and Stability Testing

Products prepared using Echinacea as a binder will be treated similarly to products containing binders such as povidone or starch. Products formulated with Echinacea will comply with full compendial monograph testing, stability indicating methods, and stability studies according to FDA and ICH regulations.

5. Conclusions

Based on the data presented several conclusions can be made.

- Echinacea has binding properties which can be utilized in the manufacture of solid pharmaceutical dosage forms;
- Binding properties were demonstrated employing various technologies: granulation, direct compression, microgranules application, etc.;
- Binding properties were demonstrated using dry (dry and wet form) and liquid extracts of Echinacea; and
- Echinacea used as an excipient in pharmaceutical dosage forms must comply with pharmacopeial requirements.

Appendix A

Pharmacopeial Forum Monographs

MONOGRAPHS (NF)

***Echinacea angustifolia*; Powdered *Echinacea angustifolia*; Powdered *Echinacea angustifolia* Extract; *Echinacea pallida*; Powdered *Echinacea pallida*; Powdered *Echinacea pallida* Extract; *Echinacea purpurea* Root; Powdered *Echinacea purpurea*; Powdered *Echinacea purpurea* Extract.** Because there are no existing *NF* monographs for these articles, the Subcommittee on Natural Products is presenting monographs for roots, powdered roots, and extracts from roots for three species of genus *Echinacea*: *E. angustifolia*, *E. pallida* and *E. purpurea*. The roots of *E. angustifolia* and *E. pallida* have been recognized internationally as medicinal plants, and articles derived from the roots of *E. purpurea* are commonly marketed as dietary supplements in the U.S.

Phenolic caffeoyl derivatives are common to all three species, and therefore a test for *Content of total phenols* is included in the monograph for each article. Whereas chicoric acid and caftaric acid are the main components of total phenols in *E. purpurea* roots, echinacoside is the main phenolic component of *E. pallida* and *E. angustifolia*. Caffeoyl derivatives also serve to distinguish *Echinacea* species from a common adulterant, *Parthenium integrifolium*, which does not contain any caffeoyl derivatives.

The three species are distinguished from one another by the presence or absence of the following components: cynarine (1,3-dicaffeoylquinic acid) and its artifact of isomerization, (1,5-dicaffeoylquinic acid) are present in *E. angustifolia*, but not in *E. pallida* or *E. purpurea*; alkamides, recognized as contributors to immunostimulant activity, are present in *E. purpurea* and particularly as dodecatetraenoic acid isobutylamides in *E. angustifolia*, but not in *E. pallida*; and ketoalkenynes and *Volatile oil content* serve as distinguishing characteristics of *E. pallida*. On the basis of these characteristics, *Thin-Layer Chromatographic Identification Tests* for the presence of caffeoyl derivatives, alkamides, and ketoalkenynes have been included in these monographs.

The Subcommittee is aware that alkamides in *E. angustifolia* consist mainly of compounds with a monoene chromophore having an absorption maximum around 212 nm, and that the most abundant component is dodecatetraenoic acid isobutylamide (a diene chromophore having an absorption maximum around 254 nm). To simplify the procedures, it is suggested that dodecatetraenoic acid isobutylamide be used as a marker at 254 nm not only for the dodecatetraenoic acid isobutylamides present in *E. angustifolia*, but also for the alkamides in *E. purpurea*. These methods use 2,4-hexadienoic acid isobutylamide as the Reference Standard. Because alkamides are not present in the roots of *E. pallida*, a test for *Content of alkylamides* is not included in the *E. pallida* monograph.

The liquid chromatographic procedures in the test for *Content of total phenols* are based on analyses performed with the Prodigy ODS-3, 100Å, 15.5% of carbon load, end-capped brand of 5-µm L1 column. Typical retention times observed for caftaric acid, chlorogenic acid, echinacoside, chicoric acid, and cynarine are about 6.8, 7.2, 10.3, 16.4, and 17.5 minutes, respectively. The liquid chromatographic procedures in the test for *Content of alkamides* are based on analyses performed with the Luna C18(2) brand of 5-µm L1 column. Typical retention times observed for dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and for dodeca-2*E*,4*E*,8Z,10Z-tetraenoic acid isobutylamide are 20.0 and 21.0 minutes, respectively.

7E00005 (NAT) RTS—30023-1

Add the following:

Echinacea angustifolia

» *Echinacea angustifolia* consists of the dried rhizome and roots of *Echinacea angustifolia* DC (Fam. Asteraceae). It is harvested in the fall after 3 or more years of growth. It contains not less than 0.5 percent of total phenols calculated on the dried basis as the sum of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), dicaffeoylquinic acids ($C_{25}H_{24}O_{12}$), and echinacoside ($C_{35}H_{46}O_{20}$). It contains not less than 0.075 per cent of dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$).

Packaging and storage—Store in well-closed, light-resistant containers.

Labeling—The label states the Latin binomial name and following the official name, the parts of the plant contained in the article.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Powdered Echinacea angustifolia Extract RS. USP 2E,4E-Hexadienoic Acid Isobutylamide RS.*

Botanic characteristics—

Macroscopic—The outer surface of the rhizome is pale to yellowish brown, crowned with remains of the aerial stem, and sometimes showing surface annulations

up to 15 mm in diameter. The roots are also pale to yellowish brown, cylindrical or slightly tapering, sometimes spirally twisted, longitudinally wrinkled and deeply furrowed, up to 4 to 10 mm in diameter, and passing imperceptibly into rhizome. The short fracture, when dry, becomes tough and pliable on exposure to air.

Microscopic—The rhizomes and roots in transverse section show a thin outer bark separated from a wide xylem by a distinct cambial line. The cork is composed of several rows of thin-walled cells containing yellowish brown pigment. The rhizome has a small circular pith, occasional small groups of thick-walled, lignified fibers in the pericycle, and a parenchymatous cortex. The phloem and xylem are composed of narrow strands of vascular tissue separated by wide, non-lignified medullary rays. Xylem vessels are lignified, 25 to 75 µm in diameter, usually with reticulate thickening but occasionally with spiral or annular thickening. Sclereids occur singly or in small groups, varying considerably in size and shape from rounded to rectangular to elongated and fiber-like, up to 300 µm long and 20 to 40 µm wide, with intercellular spaces forming schizogenous oleoresin canals that are 80 to 150 µm in diameter and contain a dense black deposit. The canals are present outside of the central cylinder only (unlike *Echinacea pallida*, where they are present both inside and outside of the central cylinder). Spherocrystalline masses of inulin occur throughout the parenchymatous tissues. Lignified fibers, 300 to 800 µm long, are present in scattered groups, and are usually surrounded by phytomelanin (unlike fibers in *Echinacea pallida*, where they usually occur singly in the periphery of the cortex and are 100 to 300 µm long, with phytomelanin often absent).

Identification—

A: *Thin Layer Chromatographic Identification Test* ⟨201⟩—

PRESENCE OF ECHINACOSIDE AND CYNARINE—

Test solution—Weigh and finely pulverize about 10 g of *Echinacea angustifolia*, and transfer 1 g of the powder to a suitable extraction thimble. Transfer the thimble to a continuous extraction apparatus, and extract for 1 hour with 50 mL of chloroform. Reserve the chloroform extract for *Identification* test *B*. Continue the extraction with 50 mL of methanol, and concentrate to a small volume at 40° under vacuum. With the aid of methanol, transfer the extract to a 10-mL volumetric flask, and dilute with methanol to volume.

Standard solution 1—Dissolve an accurately weighed quantity of USP Powdered *Echinacea angustifolia* Extract RS in methanol to obtain a solution having a concentration of about 10 mg per mL.

Standard solution 2—Dissolve an accurately weighed quantity of cynarine in methanol to obtain a solution having a concentration of about 1 mg per mL.

Developing solvent system: a mixture of ethyl acetate, formic acid, and water (17:2:1).

Spray reagent A—Dissolve a suitable quantity of 2-aminoethyl diphenylborinate in methanol to obtain a solution having a concentration of about 10 mg per mL.

Spray reagent B—Dissolve a suitable quantity of polyethylene glycol 4000 in alcohol to obtain a solution having a concentration of about 50 mg per mL.

Procedure—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* ⟨621⟩. Allow the solvent to ascend not less than 18 cm, and dry the plate in a current of air. Spray the plate with *Spray reagent A* followed by *Spray reagent B*, and examine the plate

under UV light at 365 nm: the chromatogram obtained from the *Test solution* shows a yellowish zone at an R_F value of 0.14 characteristic of echinacoside (absent or only traces present in *Echinacea purpurea*), that corresponds in color and R_F value to that in the chromatogram of *Standard solution A*, and another zone characteristic of cynarine (absent in *Echinacea pallida* and *Echinacea purpurea*) corresponding in color and R_F value (about 0.67) to that in the chromatogram of *Standard solution B*. Other colored zones of varying intensities may be observed in the chromatogram of the *Test solution*.

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

PRESENCE OF ISOBUTYLALKENYLAMIDES—

Test solution—Evaporate the chloroform extract reserved from preparation of the *Test solution* in *Identification* test *A* to dryness at 40° under vacuum. To the residue, add 1 mL of alcohol, and pass through a nylon membrane having a porosity of 0.45 μm.

Standard solution 1—Transfer an accurately weighed quantity of USP *Echinacea angustifolia* Extract RS to a centrifuge tube, and add chloroform to obtain a solution having a known concentration of about 10 mg per mL. Shake by hand to disperse, sonicate for 5 minutes, and centrifuge. Use the supernatant.

Standard solution 2—Dissolve an accurately weighed quantity of β-sitosterol in methanol to obtain a solution having a concentration of about 1 mg per mL.

Developing solvent system: a mixture of hexanes and ethyl acetate (2:1).

Spray reagent: a mixture of glacial acetic acid, sulfuric acid, and *p*-anisaldehyde (10:5:0.5).

Procedure—Proceed as directed for *Identification* test *A*. Allow the chromatogram to develop not less than 18 cm, and dry the plate in a current of air. Examine the plate under UV light at 254 nm: the chromatogram obtained from the *Test solution* shows one main zone at an R_F value of 0.5 due to 2*E*,4*E*,8*Z*,10*E*-dodecatetraenoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (absent in *E. pallida*) that corresponds in R_F value to that in the chromatogram of *Standard solution 1*, and another zone due to β-sitosterol that corresponds in R_F value to the principal spot in the chromatogram of *Standard solution 2*. Spray the plate with *Spray reagent*, and then heat the plate at 100° for 5 minutes: the chromatogram obtained from the *Test solution* shows a blue-black zone at an R_F value of 0.5 due to dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and to dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide that corresponds in R_F value to that in the chromatogram of *Standard solution 1*, and below this spot, there are several yellowish zones due to α,β-unsaturated isobutylamides (absent in *Echinacea pallida* and mostly violet in *Echinacea purpurea* due to the presence of α,β,γ,δ-unsaturated isobutylamides) that are not visible or very weak when viewed under UV light at 254 nm.

C: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that of the echinacoside peak in the chromatogram of *Standard solution 1*, as obtained in the test for *Content of total phenols*.

Microbial limits ⟨2021⟩—The total bacterial count does not exceed 10^7 per g, the total combined molds and yeast count does not exceed 10^5 per g, the coliform count is not more than 10^4 per g, the enterobacterial count is not

more than 10^4 per g, and it meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, and *Staphylococcus aureus.*

Loss on drying ⟨731⟩: not more than 10.0%.

Foreign organic matter ⟨561⟩: not more than 3.0%.

Total ash ⟨561⟩: not more than 7.0%.

Acid-insoluble ash ⟨561⟩: not more than 4.0%.

Pesticide residues ⟨561⟩: meets the requirements.

Heavy metals, *Method III* ⟨231⟩: not more than 0.001%.

Content of total phenols—

Solvent: a mixture of alcohol and water (7:3).

Solution A: a filtered and degassed solution of phosphoric acid (0.1 in 100).

Solution B: filtered and degassed acetonitrile.

Standard solution 1—Dissolve an accurately weighed quantity of USP Powdered *Echinacea angustifolia* Extract RS in *Solvent*, shaking for 1 minute, and dilute with *Solvent* to obtain a solution having a known concentration of about 1 mg of per mL. Pass through a membrane filter having a 0.45-μm or finer porosity.

Standard solution 2—Dissolve an accurately weighed quantity of USP Chlorogenic Acid RS in *Solvent*, shaking for 1 minute. Dilute quantitatively, and stepwise if necessary, with *Solvent* to obtain a solution having a known concentration of about 40 μg per mL. Pass through a membrane filter having a 0.45-μm or finer porosity.

Test solution—Transfer about 125 mg of finely powdered *Echinacea angustifolia* (capable of passing through a 40-mesh sieve), accurately weighed, to a centrifuge tube. Add 25 mL of *Solvent* and heat under reflux while shaking mechanically for 15 minutes. Centrifuge, or pass through a membrane filter having a 0.45-μm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 330-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The column temperature is maintained at 35°. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–13	90→78	10→22	linear gradient
13–14	78→60	22→40	linear gradient
14–17	60	40	isocratic
7–17.5	60→90	40→10	linear gradient
17.5–22	90	10	equilibration

Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure:* the chromatogram obtained is similar to the Reference Chromatogram for total phenols provided with USP Powdered *Echinacea angustifolia* Extract RS. Chromatograph *Standard solution 2*, and record the peak responses as directed for *Procedure:* the capacity factor, k', is not less than 3.5; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.5 %.

Procedure—Separately inject equal volumes (about 5 μL) of *Standard Solution 1*, *Standard solution 2*, and the *Test Solution* into the chromatograph, record the chromatograms, and measure the areas for the relevant peaks. Identify the relevant analytes in the chromatogram obtained from the *Test solution* by comparison with the chromatogram obtained from *Standard solution 1*. Separately calculate the percentage of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid

($C_{16}H_{18}O_9$), dicaffeoylquinic acids ($C_{25}H_{24}O_{12}$), and echinacoside ($C_{35}H_{46}O_{20}$) in the portion of *Echinacea angustifolia* taken by the formula:

$$2500F(C/W)(r_i/r_S),$$

in which F is the response factor for the relevant analyte (0.881 for caftaric acid, 0.695 for chicoric acid, 1.000 for chlorogenic acid, 0.729 for dicaffeoylquinic acids, and 2.22 for echinacoside); C is the concentration, in mg per mL, of USP Chlorogenic Acid RS in *Standard solution 2*; W is the weight, in mg, of *Echinacea angustifolia* taken; and r_i and r_S are the peak responses for the relevant analyte obtained from the *Test solution* and *Standard solution 2*, respectively. Calculate the percentage of total phenols in the portion of *Echinacea angustifolia* taken by adding the individual quantities calculated.

Content of dodecatetraenoic acid isobutylamides—

Mobile phase: a filtered and degassed mixture of acetonitrile and water (55:45).

Standard solution 1—Dissolve, with sonication, an accurately weighed quantity of USP Powdered *Echinacea angustifolia* Extract RS in methanol, shaking for 10 minutes, and dilute with methanol to obtain a solution having a concentration of about 5 mg per mL. Pass through a membrane filter having a 0.45-μm or finer porosity.

Standard solution 2—Dissolve an accurately weighed quantity of USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS in methanol, shaking for 1 minute. Dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 10 μg per mL. Pass through a membrane filter having a 0.45-μm or finer porosity.

Test solution—Transfer about 2.5 g of finely powdered *Echinacea angustifolia* (capable of passing through a 40-mesh sieve), accurately weighed, into a round-bottom flask. Add 80 mL of methanol, and reflux for 30 minutes. Cool to room temperature, and filter into a 100-mL volumetric flask, using small portions of methanol to rinse the flask and the filter. Dilute with methanol to volume, and mix. Pass through a membrane filter having a 0.45-μm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The column temperature is maintained at 30°. The flow rate is about 1.5 mL per minute. Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure:* the chromatogram obtained is similar to the Reference Chromatogram for alkamides provided with the USP Powdered *Echinacea angustifolia* Extract RS; and the resolution, R, between dodecatetraenoic acid isobutylamide peaks is not less than 1.0. Chromatograph *Standard solution 2*, and record the peak responses as directed for *Procedure:* the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Separately inject equal volumes (about 25 μL) of *Standard Solution 1*, *Standard solution 2*, and the *Test Solution* into the chromatograph, record the chromatograms, and measure the areas for the relevant peaks. Identify the peaks due to 2*E*,4*E*,8*Z*,10*E*-dodecatetraenoic acid isobutylamide and 2*E*,4*E*,8*Z*,10*Z*-dodecatetraenoic acid isobutylamide in the chromatogram obtained from the *Test solution* by comparison with the chromatogram obtained from *Standard solution 1*. Cal-

culate the percentage of dodecatetraenoic acid isobutylamides in the portion of *Echinacea angustifolia* taken by the formula:

$$10{,}000(1.353)(C/W)(r_i/r_s),$$

in which 1.353 is the response factor, F, for hexadienoic acid isobutylamide; C is the concentration, in mg per mL, of the USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS in *Standard solution 2*; W is the weight, in mg, of the portion of *Echinacea angustifolia* taken; r_i is the sum of the peak responses of the relevant analytes obtained from the *Test solution*; and r_s is the peak response obtained from *Standard solution 2*.

Botanic characteristics—Powdered *Echinacea angustifolia* is a brown powder with a slight aromatic odor and a sweet taste that quickly becomes bitter, leaving a tingling sensation on the tongue. Under a microscope, the following characteristics are observed: thin-walled polygonal cork cells with red-brown contents; lignified reticulate vessels; abundant stone cells of various shapes; fragments of oleoresin canals with reddish brown contents; and abundant thin-walled parenchyma with sphero-crystalline masses of inulin.

Other requirements—It meets the requirements of the tests for *Identification*, *Microbial limits*, *Loss on drying*, *Total ash*, *Acid-insoluble ash*, *Pesticide residues*, *Heavy metals*, *Content of total phenols*, and *Content of dodecatetraenoic acid isobutylamide* under *Echinacea angustifolia*.

Powdered *Echinacea angustifolia*—See briefing under *Echinacea angustifolia*.

7E00010 (NAT) RTS—30023-2

Add the following:

Powdered *Echinacea angustifolia*

» Powdered *Echinacea angustifolia* is *Echinacea angustifolia* reduced to a powder or very fine powder.

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—The label states the Latin binomial name and, following the official name, the part of the plant from which the article was derived.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS*. *USP Powdered Echinacea angustifolia Extract RS*.

Powdered *Echinacea angustifolia* Extract—See briefing under *Echinacea angustifolia*.

7E00015 (NAT) RTS—30023-3

Add the following:

Powdered *Echinacea Angustifolia* Extract

» Powdered *Echinacea angustifolia* Extract is prepared from *Echinacea angustifolia* roots by extraction with hydroalcoholic mixtures or other suitable solvents. The ratio of the starting crude plant material to powdered extract is between 2:1 and 8:1. It contains not less than 4.0 percent and not more than 5.0 percent of total phenols,

calculated on the dried basis as the sum of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), dicaffeoylquinic acids ($C_{25}H_{24}O_{12}$), and echinacoside ($C_{35}H_{46}O_{20}$). It contains not less than 0.6 percent of dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$).

Packaging and storage—Preserve in tight, light-resistant containers, in a cool place.

Labeling—The label states the Latin binomial name and, following the official name, the part of the plant from which the article was prepared. The label also indicates the content of total phenols, the extracting solvent or solvent mixture used for preparation, and the ratio of the starting crude plant material to powdered extract. It meets the requirements for labeling under *Botanical Extracts* ⟨565⟩.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Powdered Echinacea angustifolia Extract RS.*

Identification—

A: *Thin Layer Chromatographic Identification Test* ⟨201⟩—

Standard solution 1, *Standard solution 2*, *Developing solvent system*, *Spray reagent*, and *Procedure*—Proceed as directed for *Identification* test *B* under *Echinacea angustifolia.*

Test solution—Dissolve 0.1 g of Powdered *Echinacea angustifolia* Extract in 10 mL of methanol. Allow to stand for 15 minutes before use.

B: The retention time for the major peak in the chromatogram of the *Test solution* corresponds to that for the echinacoside peak in the chromatogram of *Standard solution 1*, as obtained in the test for *Content of total phenols.*

Microbial limits ⟨2021⟩—The total bacterial count does not exceed 10,000 per g, the total combined molds and yeasts count does not exceed 1000 per g, the coliform count does not exceed 1000 per g, and the count for enterobacteria does not exceed 1000 per g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, and *Staphylococcus aureus.*

Loss on drying ⟨731⟩: not more than 5.0%.

Heavy metals, *Method II* ⟨231⟩: 0.002%.

Organic volatile impurities, *Method I* ⟨467⟩: meets the requirements.

Content of total phenols—

Solvent, *Solution A*, *Solution B*, *Standard solution 1*, *Standard solution 2*, and *Chromatographic system*—Proceed as directed under *Echinacea angustifolia.*

Test solution—Transfer about 60 mg of Powdered Extract, accurately weighed, to a 50-mL centrifuge tube. Add 25 mL of *Solvent*, and shake by mechanical means for 15 minutes. Centrifuge, or pass through a membrane filter having a 0.45-μm or finer porosity.

Procedure—Proceed as directed for *Content of total phenols* under *Echinacea angustifolia.* Calculate the percentage of each relevant component of total phenols in the portion of Powdered Extract taken by the formula:

$$2500F(C/W)(r_i/r_s),$$

in which W is the weight, in mg, of the portion of Powdered Extract taken; and the other terms are as defined therein. Calculate the percentage of total phenols in the portion of Powdered Extract taken by adding the individual percentages.

Content of dodecatetraenoic acid isobutylamides—

Mobile phase and *Standard solution 2*—Proceed as directed for *Content of dodecatetraenoic acid isobutylamides* under *Echinacea angustifolia.*

Standard solution 1—Dissolve an accurately weighed quantity of USP Powdered *Echinacea angustifolia* Extract RS in methanol, shaking for 1 minute, and dilute with methanol to volume to obtain a solution having a known concentration of about 1 mg of extract per mL. Pass through a membrane filter having a 0.45-µm or finer porosity.

Test solution—Transfer about 500 mg of Powdered Extract, accurately weighed, to a 100-mL volumetric flask. Add 80 mL of methanol, and sonicate for 30 minutes. Dilute with methanol to volume, and pass through a membrane filter having a 0.45-µm or finer porosity.

Chromatographic system—Proceed as directed for *Content of dodecatetraenoic acid isobutylamides* under *Echinacea angustifolia.* Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure:* the chromatogram obtained is similar to the Reference Chromatogram for alkamides provided with USP Powdered *Echinacea angustifolia* Extract RS. Chromatograph *Standard solution 2*, and record the peak responses as directed for *Procedure:* the capacity factor, k', is not less than 3.5; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.5 %.

Procedure—Proceed as directed for *Content of dodecatetraenoic acid isobutylamides* under *Echinacea angustifolia.* Calculate the percentage of dodecatetraenoic acid isobutylamides in the portion of Powdered Extract taken by the formula:

$$10{,}000(1.353)(C/W)(r_i / r_S),$$

in which 1.353 is the response factor, F, for hexadienoic acid isobutylamide; W is the weight, in mg, of the portion of Powdered Extract taken; and the other terms are as defined therein.

Other requirements—It meets the requirements for *Microbial limits*, *Loss on drying*, *Heavy metals*, and *Organic volatile impurities* under Powdered *Echinacea angustifolia* Extract; and it meets the requirements for *Packaging and Storage*, *Residual Solvents*, and *Pesticide Residues* under *Botanical Extracts* ⟨565⟩.

Echinacea pallida—See briefing under *Echinacea angustifolia.*

7E00035 (NAT) RTS—23402-3

Add the following:

Echinacea pallida

» *Echinacea pallida* consists of the dried rhizome and roots of *Echinacea pallida* Nuttall (Fam. Asteraceae). It is harvested in the fall after 3 or more years of growth. It contains not less than 0.5 percent of total phenols, calculated on the dried basis as the sum of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), and echinacoside ($C_{35}H_{46}O_{20}$).

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—The label states the Latin binomial name and, following the official name, the parts of the plant contained in the article.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Echinacea pallida Extract RS.*

Botanic characteristics—

Macroscopic—The outer surface of the rhizome is pale to yellowish brown, crowned with the remains of the aerial stem, and sometimes shows surface annulations up to 15 mm in diameter. The roots are pale to yellowish brown, cylindrical or slightly tapering, sometimes spirally twisted, longitudinally wrinkled and deeply furrowed, up to 4 to 10 mm in diameter, and pass imperceptibly into rhizome. The short fracture, when dry, becomes tough and pliable on exposure to air.

Microscopic—The rhizomes and roots in transverse section show a thin outer bark separated from a wide xylem by a distinct cambial line. The cork is composed of several rows of thin-walled cells containing yellowish brown pigment. The rhizome has a small circular pith, occasional small groups of thick-walled, lignified fibers in the pericycle, and a parenchymatous cortex. The phloem and xylem are composed of narrow strands of vascular tissue separated by wide, non-lignified medullary rays. Xylem vessels are lignified, 25 to 75 μm in diameter, usually with reticulate thickening but occasionally with spiral or annular thickening. Sclereids occur singly or in small groups, varying considerably in size and shape from rounded to rectangular to elongated and fiber-like, are up to 300 μm long and 20 to 40 μm wide, with intercellular spaces forming schizogenous oleoresin canals that are 80 to 150 μm in diameter and contain a dense black deposit present both inside and outside of the central cylinder (unlike *Echinacea angustifolia*, where the canals are present only outside of the central cylinder). Spherocrystalline masses of inulin occur throughout the parenchymatous tissues. Lignified fibers, present in the periphery of the cortex, are 100 to 300 μm long and occur singly with phytomelanin often absent (unlike *Echinacea angustifolia*, where the fibers occur scattered in groups, are 300 to 800 μm long, and are usually surrounded by phytomelanin).

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

PRESENCE OF ECHINACOSIDE AND ABSENCE OF CYNARINE—

Test solution—Proceed as directed for *Identification* test *A* under *Echinacea angustifolia*, but use *Echinacea pallida* to prepare the *Test solution.*

Standard solution 1—Dissolve an accurately weighed quantity of USP Powdered *Echinacea pallida* Extract RS in methanol to obtain a solution having a known concentration of about 10 mg per mL.

Standard solution 2, *Developing solvent system*, *Spray reagent A*, and *Spray reagent B*—Proceed as directed for *Identification* test *A* under *Echinacea angustifolia.*

Procedure—Proceed as directed in the chapter. Allow the solvent to ascend not less than 18 cm, and dry the plate in a current of air. Spray the plate with *Spray reagent A* followed by *Spray reagent B*, and examine the plate under UV light at 365 nm: the chromatogram ob-

tained from the *Test solution* shows a yellowish zone at an R_F value of 0.1 characteristic of echinacoside (absent or traces in *Echinacea purpurea),* corresponding in color and R_F value to that in the chromatogram of *Standard solution A,* and does not show a zone characteristic of cynarine (present in *Echinacea angustifolia*) corresponding in color and R_F value to that in the chromatogram of *Standard solution B.* Other colored zones of varying intensities may be observed in the chromatogram of the *Test solution.*

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

PRESENCE OF KETOALKENYNES—

Test solution—Evaporate to dryness the chloroform extract reserved from preparation of the *Test solution* in *Identification* test *A* at 40° under vacuum. To the residue add 1 mL of alcohol, and pass through a nylon membrane filter having a porosity of 0.45 µm.

Standard solution 1—Transfer an accurately weighed quantity of USP *Echinacea pallida* Extract RS to a centrifuge tube, and add chloroform to obtain a solution having a concentration of about 10 mg per mL. Shake for 1 minute, and centrifuge. Use the supernatant.

Standard solution 2—Dissolve an accurately weighed quantity of β-sitosterol in methanol to obtain a solution having a concentration of about 1 mg per mL.

Developing solvent system: a mixture of toluene and ethyl acetate (7:3).

Spray reagent A: a 1% solution of vanillin in alcohol.

Spray reagent B: a 10% solution of sulfuric acid in alcohol.

Procedure—Proceed as directed for *Identification* test *A.* Spray the plate with *Spray reagent A* followed by *Spray reagent B,* and heat the plate at 120° for 3 minutes. The chromatogram obtained from the *Test solution* shows green, brown, and violet zones above the spot for β-sitosterol (R_F range 0.6 to 0.8). These zones (unlike those in *Echinacea angustifolia* and *Echinacea purpurea*) are characteristic of ketoalkenynes and correspond in R_F value to the zones obtained from the chromatogram of *Standard solution 1.*

C: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that of the echinacoside peak in the chromatogram of *Standard solution 1,* as obtained in the test for *Content of total phenols.*

Volatile oil content ⟨561⟩: between 1.0 and 2.0 mL per 100 g.

Content of total phenols—

Solvent, Solution A, Solution B, and *Standard solution 2*—Prepare as directed for *Content of total phenols* under *Echinacea angustifolia.*

Standard solution 1—Proceed as directed for *Content of total phenols* under *Echinacea angustifolia,* but use USP Powdered *Echinacea pallida* Extract RS.

Test solution—Proceed as directed for *Content of total phenols* under *Echinacea angustifolia,* but use finely powdered *Echinacea pallida.*

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed for *Content of total phenols* under *Echinacea angustifolia.* Chromatograph *Standard solution 1,* and record the peak responses as directed for *Procedure:* the chromatogram obtained is similar to the Reference Chromatogram for total phenols provided with the USP *Echinacea pallida* Extract RS. Chromatograph *Standard solution 2,* and record the peak re-

sponses as directed for *Procedure:* the capacity factor, k', is not less than 3.5; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.5%.

Procedure—Proceed as directed for *Content of total phenols* under *Echinacea angustifolia.* Separately calculate the percentage of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), and echinacoside ($C_{35}H_{46}O_{20}$) in the portion of *Echinacea pallida* taken by the formula:

$$2500F(C/W)(r_i/r_S),$$

in which F is the response factor for each relevant analyte (0.881 for caftaric acid, 0.695 for chicoric acid, 1.000 for chlorogenic acid, and 2.22 for echinacoside); C is the concentration, in mg per mL, of USP Chlorogenic Acid RS in *Standard solution 2; W* is the weight, in mg, of *Echinacea pallida* taken; and r_i and r_S are the peak responses for the relevant analyte obtained from the *Test solution* and *Standard solution* 2, respectively. Calculate the percentage of total phenols in the portion of *Echinacea pallida* taken by adding the individual quantities calculated.

Other requirements—It meets the requirements for *Microbial limits, Loss on drying, Foreign organic matter, Total ash, Acid-insoluble ash, Pesticide residues,* and *Heavy metals* under *Echinacea angustifolia.*

Powdered *Echinacea pallida*—See briefing under *Echinacea angustifolia.*

7E00037 (NAT) RTS—24402-4

Add the following:

Powdered *Echinacea pallida*

» Powdered *Echinacea pallida* is *Echinacea pallida* reduced to a powder or very fine powder.

Packaging and storage—Preserve in tight, light-resistant containers.

Labeling—The label states the Latin binomial name and, following the official name, the part of the plant from which the article was derived.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Powdered Echinacea pallida Extract RS.*

Botanic characteristics—Powdered *Echinacea pallida* is a brown powder with a faint aromatic odor and a slightly acrid, persistent taste. It turns bright yellow when mounted in sodium hydroxide solution. Under a microscope, the following characteristics are observed: groups of secretory canals with brown contents, surrounded by parenchymatous cells containing cluster crystals of calcium oxalate; and parenchymatous cells with small starch granules, thick-walled lignified fibers, and fragments of reticulate and pitted vessels.

Other requirements—It meets the requirements of the tests for *Identification, Microbial limits, Loss on drying, Total ash, Acid-insoluble ash, Volatile oil content, Pesticide residues, Heavy metals,* and *Content of total phenols* under *Echinacea pallida.*

Powdered *Echinacea pallida* Extract—See briefing under *Echinacea angustifolia*.

7E00041 (NAT) RTS—24402-5

Add the following:

Powdered *Echinacea pallida* Extract

» Powdered *Echinacea pallida* Extract is prepared from *Echinacea pallida* roots by extraction with hydroalcoholic mixtures or other suitable solvents. The ratio of the starting crude plant material to powdered extract is between 2:1 and 8:1. It contains not less than 4.0 percent and not more than 5.0 percent of total phenols, calculated on the dried basis as the sum of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), dicaffeoylquinic acids ($C_{25}H_{24}O_{12}$), and echinacoside ($C_{35}H_{46}O_{20}$).

Packaging and storage—Preserve in tight, light-resistant containers, in a cool place.

Labeling—The label states the Latin binomial name and, following the official name, the parts of the plant from which the article was prepared. The label also indicates the content of total phenols, the extracting solvent or solvent mixture used for preparation, and the ratio of the starting crude plant material to powdered extract. It meets the requirements for labeling under *Botanical Extracts* ⟨565⟩.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Powdered Echinacea pallida Extract RS.*

Identification—

A: *Thin Layer Chromatographic Identification Test* ⟨201⟩—

Standard solution 1, *Standard solution 2*, *Developing solvent system*, *Spray reagent A*, *Spray reagent B*, and *Procedure*—Proceed as directed for *Identification* test *A* under *Echinacea pallida*.

Test solution—Dissolve 0.1 g of Powdered Extract in 10 mL of methanol. Allow to settle before use.

B: The retention time for the major peak in the chromatogram of the *Test solution* corresponds to that of the echinacoside peak in the chromatogram of *Standard solution 1*, as obtained in the test for *Content of total phenols*.

Content of total phenols—

Solvent, *Solution A*, *Solution B*, *Standard solution 1*, *Standard solution 2*, and *Chromatographic system*—Proceed as directed for *Content of total phenols* under *Echinacea pallida*.

Test solution—Transfer about 60 mg of Powdered Extract, accurately weighed, to a 50-mL centrifuge tube. Add 25 mL of *Solvent*, and shake by mechanical means for 15 minutes. Centrifuge, or pass through a membrane filter of 0.45-μm or finer porosity.

Procedure—Proceed as directed for *Content of total phenols* under *Echinacea pallida*. Separately calculate the percentage of each relevant component of total phenols in the portion of Powdered Extract taken by the formula:

$$2500F(C/W)(r_i/r_s),$$

in which W is the weight, in mg, of the portion of Powdered Extract taken, and the other terms are as defined therein. Calculate the percentage of total phenols in the

portion of Powdered Extract taken by adding the individual quantities calculated.

Other requirements—It meets the requirements for *Microbial limits*, *Loss on drying*, *Heavy metals*, and *Organic volatile impurities* under Powdered *Echinacea angustifolia* Extract, and it meets the requirements for *Packaging and Storage*, *Residual Solvents*, and *Pesticide Residues* under *Botanical Extracts* ⟨565⟩.

***Echinacea purpurea* Root**—See briefing under *Echinacea angustifolia*.

7E00060 (NAT) RTS—30094-1

Add the following:

Echinacea purpurea Root

» *Echinacea purpurea* Root consists of the dried rhizome and roots of *Echinacea purpurea* (L.) Moench (Fam. Asteraceae). It is harvested in the fall after 3 or more years of growth. It contains not less than 0.5 percent of total phenols calculated on the dried basis as the sum of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), and echinacoside ($C_{35}H_{46}O_{20}$). It contains not less than 0.025 percent of alkamides calculated as dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$).

Packaging and storage—Store in well-closed, light-resistant containers.

Labeling—The label states the Latin binomial name and, following the official name, the parts of the plant contained in the article.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Echinacea purpurea Extract RS. USP 2E,4E-Hexadienoic Acid Isobutylamide RS.*

Botanic characteristics—

Macroscopic—The roots are cylindrical and irregularly branched. The outer surface is dark brown and longitudinally striated; fractures are short and tough. Transverse sections show a thin periderm and yellowish xylem with distinct rays. In older roots, the pith is spongy, with a brownish center surrounded by yellow.

Microscopic—Rhizomes and roots in transverse section show a thin outer bark separated from a wide xylem by a brown vascular cambium. The cork is composed of several rows of thin-walled cells containing brown pigment. Schizogenous resin canals are present in the cortex. The rhizome contains bast fibers and stone cells. The xylem, with distinct rays, contains tracheary elements composed of reticulated vessels and tracheids (about 80 × 30 µm) with bordered pits and slanted end walls. Vessels and tracheids are surrounded by thick-walled parenchyma and fibers; fibers are elongated with narrow lumens and funnel-shaped ends (20 to 40 µm wide). Polygonal sclereids (about 50 µm in diameter) are also present. Xylem fibers have minimal or no phytomelanin deposits (unlike *Echinacea angustifolia* and *Echinacea pallida*). A melanogenic layer is present between adjacent xylem parenchyma cell walls. The rhizome, with pith, is composed of pitted parenchyma cells containing inulin crystals. Starch is minimal to absent, and calcium oxalate crystals are absent.

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

PRESENCE OF CHICORIC ACID AND ABSENCE OF ECHINACOSIDE—

Test solution, *Standard solution 1*, *Standard solution 2*, *Developing solvent system*, *Spray reagent A*, and *Spray Reagent B*—Prepare as directed for *Identification* test *A* under *Echinacea angustifolia*, but use *Echinacea purpurea* Root to prepare the *Test solution*, and use USP Powdered *Echinacea purpurea* Extract RS to prepare *Standard solution 1*.

Procedure—Proceed as directed in the chapter. Allow the chromatogram to ascend not less than 18 cm, and dry the plate in a current of air. Spray the plate with *Spray reagent A* followed by *Spray reagent B*, and examine the plate under UV light at 365 nm: the chromatogram obtained from the *Test solution* shows a yellowish green zone at an R_F value of 0.75 due to chicoric acid and another yellowish green zone at an R_F value of 0.45 due to caftaric acid, both zones corresponding in color and R_F value to zones in the chromatogram of *Standard solution 1*. The chromatogram obtained from the *Test solution* does not show or shows only traces of a zone at an R_F value of 0.1 due to echinacoside (present in *Echinacea angustifolia* and in *Echinacea pallida*) that corresponds to a yellowish spot in the chromatogram of *Standard solution 1*, and does not show a zone that corresponds in color and R_F value to the spot for cynarine (present in *Echinacea angustifolia*) obtained from *Standard solution 2*. Other colored zones of varying intensities may be observed in the chromatogram of the *Test solution*.

B: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

PRESENCE OF ISOBUTYLALKENYLAMIDES—

Test Solution, *Standard solution 2*, *Developing solvent system*, and *Spray reagent*—Proceed as directed for *Identification* test *B* under *Echinacea angustifolia*, but use the chloroform extract from *Identification* test *A* under *Echinacea pupurea Root* to prepare the *Test solution*.

Standard solution 1—Dissolve an accurately weighed quantity of USP *Echinacea purpurea* Extract RS in methanol to obtain a solution having a known concentration of about 10 mg per mL.

Procedure—Proceed as directed in the chapter. Allow the solvent to ascend not less than 18 cm, and dry the plate in a current of air. Examine the plate under UV light at 254 nm: the chromatogram obtained from the *Test solution* shows one zone corresponding in R_F value to the zone due to β-sitosterol in the chromatogram of *Standard solution 2*, and one main zone corresponding in R_F value to the zone due to dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide in the chromatogram of *Standard solution 1*. Spray the plate with *Spray reagent*, and then heat the plate at 100° for 5 minutes. Examine the plate under long wavelength UV light: the zone due to dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide turns blue-black, and below this zone there are several other zones due to α,β,γ,δ-unsaturated isobutylamides (not detectable in *Echinacea pallida*) that turn violet (unlike the corresponding zones in the chromatogram of *Echinacea angustifolia* that are mostly yellowish due to α,β-unsaturated isobutylamides).

C: The retention times for the relevant peaks in the chromatogram of the *Test solution*, mainly due to caftaric acid and chicoric acid, correspond to those in the chromatogram of *Standard solution 1*, as obtained in the test for *Content of total phenols*. An echinacoside peak is not detectable or very weak.

Content of total phenols—

Solvent, *Solution A*, *Solution B*, *Standard solution 2*, and *Test solution*—Proceed as directed in the test for *Content of total phenols* under *Echinacea angustifolia*, but use finely powdered *Echinacea purpurea* Root to prepare the *Test solution*.

Standard solution 1—Dissolve an accurately weighed quantity of USP Powdered *Echinacea purpurea* Extract RS in *Solvent*, shaking for 1 minute, and dilute with *Solvent* to obtain a solution having a known concentration of about 5 mg per mL. Pass through a membrane filter having a 0.45-µm or finer porosity.

Chromatographic system (see *Chromatography* ⟨621⟩)—Proceed as directed in the test for *Content of total phenols* under *Echinacea angustifolia*. The chromatograph is programmed as follows:

Time (Minutes)	*Solution A* (%)	*Solution B* (%)	Elution
0–13	90→78	10→22	linear gradient
13–14	78→60	22→40	linear gradient
14–17.5	60	40	isocratic
17.5–18	60→90	40→10	linear gradient
18–30	90	10	equilibration

Chromatograph *Standard solution 1*, and record the peak responses as directed for *Procedure:* the chromatogram obtained is similar to the Reference Chromatogram for total phenols provided with the USP *Echinacea purpurea* Extract RS. Chromatograph *Standard solution 2*, and record the responses as directed for *Procedure:* the relative standard deviation for replicate injections of *Standard solution 2* is not more than 2%.

Procedure—Proceed as directed in the test for *Content of total phenols* under *Echinacea angustifolia*. Separately calculate the percentage of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), and echinacoside ($C_{35}H_{46}O_{20}$) in the portion of *Echinacea purpurea* Root taken by the formula:

$$2500F(C/W)(r_i/r_S),$$

in which F is 0.881 for 2-*O*-caffeoyl tartaric acid, 0.695 for chicoric acid, 1.000 for chlorogenic acid and 2.220 for echinacoside; C is the concentration, in mg per mL, of USP Chlorogenic Acid RS in *Standard solution 2*; W is the weight, in mg, of *Echinacea purpurea* Root taken; and r_i and r_S are the peak responses for the relevant analyte obtained from the *Test solution* and *Standard solution 2*, respectively. Calculate the percentage of total phenols in the portion of *Echinacea purpurea* Root taken by adding the individual quantities calculated.

Content of alkamides—

Mobile phase, *Standard solution 1*, *Standard solution 2*, *Test solution*, and *Chromatographic system*—Proceed as directed in the test for *Content of dodecatetraenoic acid isobutylamides* under *Echinacea angustifolia*, but use USP Powdered *Echinacea purpurea* Extract RS to prepare *Standard solution 1*, use *Echinacea purpurea* Root to prepare the *Test solution*, and for the *Chromatographic system* use the Reference Chromatogram for alkamides provided with the USP Powdered *Echinacea purpurea* Extract RS.

Procedure—Proceed as directed in the test for *Content of dodecatetraenoic acid isobutylamides* under *Echinacea*

angustifolia. Identify the peaks of the ten major alkamides in the chromatogram obtained from the *Test solution* by comparison with the chromatogram obtained from *Standard solution 1.* Calculate the percentage of alkamides in the portion of *Echinacea purpurea* Root taken by the formula:

$$10(1.353)(C/W)(r_i/r_s),$$

in which 1.353 is the response factor for hexadienoic acid isobutylamide; C is the concentration, in mg per mL, of USP 2*E*,4*E*-Hexadienoic Acid Isobutylamide RS in *Standard solution 2;* W is the weight, in g, of *Echinacea purpurea* Root taken; r_i is the sum of the peak responses of the relevant analytes obtained from the *Test solution;* and r_s is the peak response obtained from *Standard solution 2.*

Other requirements—It meets the requirements for *Microbial limits, Loss on drying, Foreign organic matter, Total ash, Acid-insoluble ash, Pesticide residues,* and *Heavy metals* under *Echinacea angustifolia.*

Powdered *Echinacea purpurea*—See briefing under *Echinacea angustifolia.*

7E00065 (NAT) RTS—30094-2

Add the following:

Powdered *Echinacea purpurea*

» Powdered *Echinacea purpurea* is *Echinacea purpurea* Root reduced to a powder or very fine powder.

Packaging and storage—Preserve in well-closed, light-resistant containers.

Labeling—The label states the Latin binomial name and, following the official name, the part of the plant from which the article was derived.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Powdered Echinacea purpurea Extract RS.*

Botanic characteristics—Under a microscope, the following characteristics are observed: vessels (80 × 30 μm) with slanted end walls and spiral or pitted secondary walls; rectangular cork cells (150 × 60 μm) with brown inclusions; rectangular parenchymatous cells (120 × 30 μm), some pitted; elongated fiber cells having a narrow lumen with funnel-shaped end (20 to 40 μm wide); polygonal sclereids; a melanogenic layer of variable thickness, interspersed between the cell walls of the parenchyma; and lignified sclereids, vessels, and fibers. Starch is present; calcium oxalate and inulin crystals are absent.

Other requirements—It meets the requirements of the tests for *Identification, Microbial limits, Loss on drying, Total ash, Acid-insoluble ash, Pesticide residues, Heavy metals, Content of total phenols,* and *Content of alkamides* under *Echinacea purpurea Root.*

Powdered *Echinacea purpurea* Extract—See briefing under *Echinacea angustifolia.*

7E00070 (NAT) RTS—30094-3

Add the following:

Powdered *Echinacea purpurea* Extract

» Powdered *Echinacea purpurea* Extract is prepared from dried *Echinacea purpurea* Root by extraction with hydroalcoholic mixtures or other suitable solvents. The ratio of the starting crude plant material to Powdered Extract is between 2:1 and 8:1. It contains not less than 4.0 percent of total phenols calculated on the dried basis as the sum of caftaric acid ($C_{13}H_{12}O_9$), chicoric acid ($C_{22}H_{18}O_{12}$), chlorogenic acid ($C_{16}H_{18}O_9$), and echinacoside ($C_{35}H_{46}O_{20}$). It contains not less than 0.025 percent of alkamides calculated on the dried basis as dodecatetraenoic acid isobutylamides ($C_{16}H_{25}NO$).

Packaging and storage—Preserve in tight, light-resistant containers, in a cool place.

Labeling—The label states the Latin binomial name and, following the official name, the parts of the plant from which the article was prepared. The label also indicates the content of total phenols, the extracting solvent or solvent mixture used for preparation, and the ratio of the starting crude plant material to Powdered Extract. It meets the requirements for labeling under *Botanical Extracts* ⟨565⟩.

USP Reference standards ⟨11⟩—*USP Chlorogenic Acid RS. USP Powdered Echinacea purpurea Extract RS.*

Identification—

A: *Thin-Layer Chromatographic Identification Test* ⟨201⟩—

Test solution, Standard solution 1, Standard solution 2, Developing solvent system, Spray reagent, and *Procedure*—Proceed as directed for *Identification* test *B* under *Echinacea purpurea Root,* but use Powdered *Echinacea purpurea* Extract to prepare the *Test solution.*

B: The retention times of the peaks for chicoric and caftaric acids in the chromatogram of the *Test solution* correspond to those in the chromatogram of *Standard solution 1,* as obtained in the test for *Content of total phenols.*

Content of total phenols—

Solvent, Solution A, Solution B, Standard solution 1, Standard solution 2, and *Chromatographic system*—Proceed as directed for *Content of total phenols* under *Echinacea purpurea Root.*

Test solution—Proceed as directed under Powdered *Echinacea angustifolia* Extract, but use Powdered *Echinacea purpurea* Extract.

Procedure—Proceed as directed in the test for *Content of total phenols* under *Echinacea purpurea Root.* Calculate the percentage of each relevant component of total phenols in the portion of Powdered Extract taken by the formula:

$$2500F(C/W)(r_i/r_S),$$

in which W is the weight, in mg, of the portion of Powdered Extract taken, and the other terms are as defined therein. Calculate the percentage of total phenols in the portion of Powdered Extract taken by adding the individual percentages.

Content of alkamides—

Mobile phase, *Standard solution 1*, *Standard solution 2*, *Test solution*, and *Chromatographic system*—Proceed as directed in the test for *Content of alkamides* under *Echinacea purpurea Root*, but use Powdered *Echinacea purpurea* Extract to prepare the *Test solution*.

Procedure—Proceed as directed in the test for *Content of alkamides* under *Echinacea purpurea Root*. Calculate the percentage of alkamides in the portion of Powdered Extract taken by the formula:

$$10(1.353)(C/W)(r_i/r_s),$$

in which 1.353 is the response factor for 2*E*,4*E*-hexadienoic acid isobutylamide; *W* is the weight, in g, of the portion of Powdered Extract taken; and the other terms are as defined therein.

Other requirements—It meets the requirements for *Microbial limits*, *Loss on drying*, *Heavy metals*, and *Organic volatile impurities* under *Powdered Echinacea angustifolia Extract*, and it meets the requirements for *Packaging and Storage*, *Residual Solvents* and *Pesticide Residues* under *Botanical Extracts* ⟨565⟩.

Eleuthero; Powdered Eleuthero; Powdered Eleuthero Extract. Because there are no existing *NF* monographs for these articles, new monographs are being previewed. The reversed phase liquid chromatographic procedure for the *Content of eleutherosides B and E* test are based on analyses performed with the Nucleosil RP18 brand of L1 column. Typical retention times for eleutheroside B and eleutheroside E are 10.5 and 20.0 minutes, respectively.

7E00200 (NAT) RTS—23692-3

Add the following:

Eleuthero

» Eleuthero is the dried rhizome with roots *Eleutherococcus senticosus* (Rupr. et Maxir (Fam. Araliaceae) [*Acanthopanax senticos* Harms]. It contains not less than 0.08 perce of the sum of eleutheroside B and eleuther side E, calculated on the dried basis.

Packaging and storage—Preserve in well-closed co tainers, protected from light.

Labeling—The label states the Latin binomial nan and, following the official name, the parts of the pla contained in the article.

USP Reference standards ⟨11⟩—*USP Powdered Ele uthero Extract RS*.

Botanic characteristics—

Macroscopic—The rhizome is knotty and of irregular cylindrical form with a diameter of 15 to 40 mm. The heartwood area is light brown, and the connecting splint wood is pale yellow. The bark is approximately 2 mm thick and is firmly affixed to the xylem. The surface is gray-brown or black-brown, coarse, and longitudinally valleculate and plicate. A broken rhizome particularly inside of the xylem is coarse and fibrous. The fractured surface of the bark shows short thin fibers. Numerous roots spring from the underside of the rhizome. These roots are 35 to 150 mm long, cylindrical, and knotty, with a diameter of 3 to 15 mm. The surface of the root is gray-brown to black-brown, is smoother than the rhizome, and has longitudinal stripes. A 0.5-mm thin bark is tightly affixed to the pale yellow xylem. A broke

Appendix B

Certificate of Analysis

Extraits de plantes pour l'industrie pharmaceutique et alimentaire
Plant extracts for Pharmaceuticals and for Health Food

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CERTIFICATE OF ANALYSIS

Extractum Siccum — ECHINACEA PURPUREA E HB

Article-Nr. 50841.01

Botanical Name	Echinacea purpurea Moench.
Plant Material	herba (fresh)
Drug:Extract Ratio	**40-50:1**
Description	brown, fine powder
Identity	
Loss on drying	7.1 %
Content of Essential Oils	
Content of Active Substance	**β-1,2-D-Fructofuranosid/Fructose: 5.7 % HPLC**
Bulk Density	430 g / l
Solubility in water	
Microbiology	
- Aerobic bacteria	< 100 / g
- Fungi	< 10 / g
- Enterobacteries and other gram-neg. bact.	< 10 / g
- Escherichia Coli	complies
- Staphylococcus Aer.	complies
- Salmonella	complies
Pesticide Residues according to Data-Sheet	complies to Purity Data Sheet
Heavy Metals	complies
Extraction medium	Press-juice
Radioactivity (Cs134 + Cs137)	< 600 Bq/kg
Carrier	none
Preserving Agent	none
Exp-Date	08/2001

Ziefen, January 19, 1999

Georg Wolfgang / Analytical Department
PHARMABETA LTD Ziefen/Switzerland